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Effect of Interferon on GB Virus C and Hepatitis C Virus in Hepatitis Patients With the Co-Infection

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Of 74 patients who were infected with hepatitis C virus (HCV) and received interferon, 12 (16%) were positive for RNA of GB virus C (GBV-C). RNA of GBV-C was determined in sera from the co-infected patients retrospectively, and the effect of interferon on GBV-C was compared with that on HCV in them. Titers of both GBV-C and HCV RNAs decreased during interferon in all of them. Two patients lost both GBV-C and HCV RNAs and remained clear until 6 months after treatment with interferon, while 2 lost RNA for GBV-C only and 2 for HCV RNA alone. Low pretreatment RNA titers of GBV-C and HCV correlated with the efficacy of interferon in clearing. Alanine aminotransferase returned to normal only in the patients who lost HCV RNA, regardless of the persistence or loss of GBV-C RNA. These results indicate that the response to interferon of GBV-C is comparable to but independent of that of HCV and that the persistence of GBV-C would not prevent the normalization of aminotransferases in response to interferon in patients with chronic hepatitis C. ***J. Med. Virol. 52:156–160, 1997.*** © 1997 Wiley-Liss, Inc.

KEY WORDS: hepatitis viruses; hepatitis C virus; chronic hepatitis; cirrhosis; interferon

INTRODUCTION

The ability to diagnose hepatitis C virus (HCV) infection in patients with chronic non-A, non-B hepatitis indicates that HCV is the major etiologic agent of this disease [Houghton et al., 1991]. However, there remain some patients with cryptogenic hepatitis who are without markers of known hepatitis viruses and in whom the infection with non-A to E hepatitis viruses is postulated [Alter and Bradley, 1995].

Recently, viral agents presumably responsible for non-A to E hepatitis have been reported independently by 2 groups of investigators. Simons et al. [1995] have cloned GB virus C (GBV-C) from sera of symptom-free carriers and hepatitis patients in the United States, Canada, and Africa, and Linnen et al. [1996] discovered hepatitis G virus (HGV) in serum from a patient with chronic hepatitis in the United States. GBV-C and HGV are positive stranded RNA viruses of approximately 9400 nucleotides, and each has a genomic organization similar to that of the *Flaviviridae.* Although they both show sequence similarity to HCV, they are too divergent to be classified as genotypes of HCV [Simons et al., 1995; Leary et al., 1996; Linnen et al., 1996]. GBV-C and HGV share 86% of nucleotide sequence and 96% or more of amino acid sequence, and therefore they are presumed to be different isolates of the same virus. The exact hepatitis-inducing activity of GBV-C/HGV is not clear, and their response to interferon (IFN) is not known.

GBV-C/HGV tends to co-infect with HCV [Simons et al., 1995; Linnen et al., 1996; Masuko et al., 1996; Tsuda et al., 1996]. Of 74 patients with chronic hepatitis C who received IFN, 12 were found to be co-infected with GBV-C in a retrospective analysis. Determination and titration of GBV-C RNA in their sera during and after IFN provided an opportunity for evaluating the response to IFN of GBV-C with reference to that of HCV and normalization of aminotransferases.

PATIENTS AND METHODS

Patients

Until September 1995, 74 patients with chronic hepatitis C received IFN and were followed for 6 months or longer thereafter. They included 50 males and 24 females aged 46.0 ± 11.8 years (range: 24–69 years). Laparoscopic or echo-guided liver biopsies were undertaken, and histological diagnosis was based on the criteria of de Groote et al. [1968]. Chronic persistent hepatitis (CPH) was diagnosed in 30, chronic active hepatitis of 2A category (CAH2A) in 31, chronic active hepatitis of 2B category (CAH2B) in the remaining 13. Patients infected with hepatitis B virus (HBV)

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were excluded, and none of the patients had antibodies to human immunodeficiency virus type 1.

The pretreatment sera and sera sampled during IFN and at least until 6 months after the completion of IFN had been stored at −80°C. HCV RNA was titrated and levels of transaminases were determined. Patients who were found to be infected with GBV-C before IFN therapy were followed also for GBV-C RNA and titers were measured.

IFN Therapy

IFN α2a (Roferon-A, Nippon Roche K.K., Tokyo, Japan) was given to 54 patients, IFN α2b (Intron-A, Schering-Plough, Osaka, Japan) to 3 patients, natural IFN α (Sumiferon, Sumitomo Pharmaceutical Co., Osaka, Japan) to 16 patients, and natural IFN β (Feron, Toray Industries, Tokyo, Japan) to the remaining patient. Each received 3–9 million units (MU) of IFN daily for 2 weeks and then 3 times a week for at least 22 weeks, with a total dose of 672 ± 205 MU (range: 234–1404 MU). The clinico-chemical response to IFN was judged by the normalization of alanine aminotransferase levels (4–17 IU/L) which was required to persist at least until 6 months after the completion of therapy.

Detection of GBV-C RNA

GBV-C RNA was determined in RNAs extracted from 100 μl of test serum by reverse transcription (RT)-polymerase chain reaction (PCR) with nested primers deduced from conserved areas in the 5′ untranslated region (UTR) of reported GBV-C/HGV genomes by the method reported previously [Shimizu et al., 1996]. Serial tenfold dilutions of extracted RNAs were made in dH_2O supplemented with 20 μg/ml glycogen (Boehringer Mannheim, Mannheim, Germany), and they were tested for GBV-C RNA to determine the highest dilution in which the viral RNA was detectable. The result was converted to represent the titer (10^N) per ml of the test serum. The titration was carried out in duplicate for each serum. When the 2 test results did not agree, a third test was undertaken and the consensus of 3 determinations was adopted. Standard samples containing 2 known concentrations of GBV-C RNA (10^2/ml and 10^5/ml) were tested in parallel in each assay, and it was repeated when the expected titers were not obtained. PCR was performed in stringent conditions under the guidelines of Kwok and Higuchi, [1989], with 1 positive control and 2 negative controls inserted every 20 samples.

Serological Markers of HCV and HBV Infections

Hepatitis B surface antigen (HBsAg) was determined by passive hemagglutination with commercial kits (MyCell, Institute of Immunology Co., Ltd., Tokyo, Japan) and antibody to HCV (anti-HCV) by enzyme-linked immunosorbent assay of the second generation (Ortho ELISA II, Ortho Diagnostic Systems, Tokyo, Japan) with an absorbance at 450 nm exceeding 0.65 considered reactive.

RNAs were extracted from 100 μl of test serum, and HCV RNA was determined by RT-PCR with nested primers deduced from the 5′UTR [Okamoto et al., 1994]. HCV RNA was semi-quantified by the method described above for the titration of GBV-C RNA. Genotypes of HCV were determined by selective amplification by a second-generation PCR method with type-specific sense as well as antisense primers deduced from the core gene [Okamoto et al., 1996]. The results were recorded by a mixed terminology system of Okamoto et al. [1993] and Simmonds et al. [1993], such as I/1a, II/1b, III/2a, IV/2b, and V/3a.

TABLE I. Characteristics of Patients With Chronic Hepatitis C Who Were Co-infected With GB Virus C and Who Were Not

Features	GBV-C RNA Positive (n = 12)	GBV-C RNA Negative (n = 62)	Differences
Age (yrs)	52.5 ± 9.0	44.7 ± 12.0	$P < 0.05$
Male	10 (83%)	40 (65%)	
Transfusion	4 (33%)	21 (34%)	
Liver disease[a]			
CPH	5 (42%)	25 (40%)	
CAH2A	5 (42%)	26 (42%)	
CAH2B	2 (17%)	11 (18%)	
ALT (IU/L)	72 ± 69	95 ± 69	
HCV RNA			
$\geq 10^6$/ml	2 (17%)	28 (45%)	
10^4–10^5 ml	3 (25%)	32 (52%)	
$\leq 10^3$/ml	7 (58%)	2 (3%)	
HCV genotypes			
II/1b	4 (33%)	42 (68%)	
III/2a	6 (50%)	16 (26%)	
IV/2b	1 (8%)	3 (6%)	
V/3a	0	1 (2%)	
Unclassifiable	1 (8%)	0	
Response to interferon	4 (33%)	19 (31%)	

[a]CPH, chronic persistent hepatitis; CAH2A, chronic active hepatitis of 2A category; CAH2B, CAH of 2B category.

Statistical Analysis

Frequencies between groups were compared using the Fisher's exact test and χ^2 test, and group means were compared using Student's *t*-test.

RESULTS

Infection With GBV-C in Patients With Chronic Hepatitis C

Of the 74 patients with chronic hepatitis C who received IFN, 12 (16%) were positive for serum GBV-C RNA. Table I compares features of the 12 patients with chronic hepatitis C who were co-infected with GBV-C and features of the remaining 62 patients who were not. The patients co-infected with GBV-C and HCV were older than those who were not ($P < 0.05$). Other than that, there were no appreciable differences between the two groups.

A history of transfusion was reported by 33% of co-infected patients and 34% of those who were not. HCV RNA titers $\geq 10^6$/ml were found in only 17% of co-infected patients, considerably less frequently than in

TABLE II. Response to Interferon of RNAs of GB Virus C and Hepatitis C Virus, as Well as the Normalization of Alanine Aminotransferase, in the 12 Patients Co-infected With Both Viruses*

Case no.	Age and sex	Diagnosis[a]	Risk factors	Interferon Kind[c]	Interferon Dose (MU)	GBV-C RNA Before (10^N/ml)	GBV-C RNA +6M (10^N/ml)	HCV RNA Before (10^N/ml)	HCV RNA +6M (10^N/ml)	HCV RNA Genotype	ALT[b] Before (IU/L)	ALT[b] +6M (IU/L)
1	54M	CAH2A	—	α2a	756	1	—	1	—	III/2a	71	16
2	45M	CPH	—	α2a	432	≥4	—	1	—	III/2a	18	17
3	51M	CAH2A	—	α2a	774	1	—	≥4	≥4	III/2a	26	61
4	54M	CAH2B	Transfusion	α	612	1	—	3	≥4	IV/2b	272	202
5	60M	CPH	Tattooing	α2b	840	3	≥4	1	—	II/1b	43	10
6	58M	CAH2A	Transfusion	α2a	756	≥4	≥4	2	—	III/2a	39	14
7	47M	CPH	—	α2a	1188	2	1	≥4	≥4	II/1b	33	43
8	54M	CAH2A	—	α	612	1	1	2	3	II/1b	89	61
9	69M	CAH2A	Transfusion	α2a	297	≥4	≥4	≥4	≥4	II/1b	113	127
10	57F	CAH2B	Transfusion	α	558	3	≥4	≥4	≥4	III/2a	76	33
11	35M	CPH	Operation	α2a	756	3	≥4	3	3	UC[d]	57	65
12	42F	CPH	—	α2a	756	3	≥4	≥4	≥4	III/2a	26	23

*The response was evaluated at 6 months after the completion of interferon (+6M).
[a]CPH, chronic persistent hepatitis; CAH2A, chronic active hepatitis of 2A category; CAH2B, CAH of 2B category.
[b]Normal values ranged from 4 to 17 IU/L.
[c]α2a, recombinant interferon α2a; α, natural interferon α.
[d]Unclassifiable.

those who were not (45%); the difference fell short of being significant, however. Infection with HCV of genotype II/1b occurred in 33% of the co-infected patients, somewhat less frequently than those who were not (68%).

The 12 patients co-infected with GBV-C received 695 ± 223 MU of IFN during 26.1 ± 1.2 weeks, which compared with 668 ± 203 MU and 26.4 ± 1.2 weeks for the 62 patients who were infected with HCV only. IFN α2a was given to 67% of co-infected patients, as compared to 74% of patients with HCV infection alone. Response to IFN, judged by normalization of ALT levels persisting for at least 6 months after the completion of therapy, was no different between the 2 groups of patients. It was achieved by 33% of co-infected patients and 31% of those who were not.

Response to IFN of GBV-C and HCV RNAs in the 12 Patients Who Were Co-Infected

Various features of the 12 patients who were co-infected with GBV-C and HCV and received IFN are listed in Table II. Titers of GBV-C and HCV RNAs and normalization of ALT levels during and after IFN are illustrated in Figure 1. At the completion of IFN, 9 lost GBV-C RNA and 10 lost HCV RNA. At 6 months after IFN, however, only 4 remained clear of GBV-C RNA and 4 stayed negative for HCV RNA; two were persistently negative for both GBV-C and HCV RNAs. Normalization of ALT persisting for at least 6 months after IFN was observed only in the 4 patients who became negative for HCV RNA. ALT levels did not normalize in the 2 patients who lost GBV-C RNA but retained HCV RNA.

The 4 patients who remained clear of GBV-C had low pretreatment titers of GBV-C RNA; 3 had titers at 10^1/ml. However, a single patient (case 2) with a high pretreatment titer (≥ 10^4/ml) lost GBV-C RNA persistently, while another (case 8) with a low pretreatment titer (10^1/ml) lost it during IFN but turned positive after the therapy.

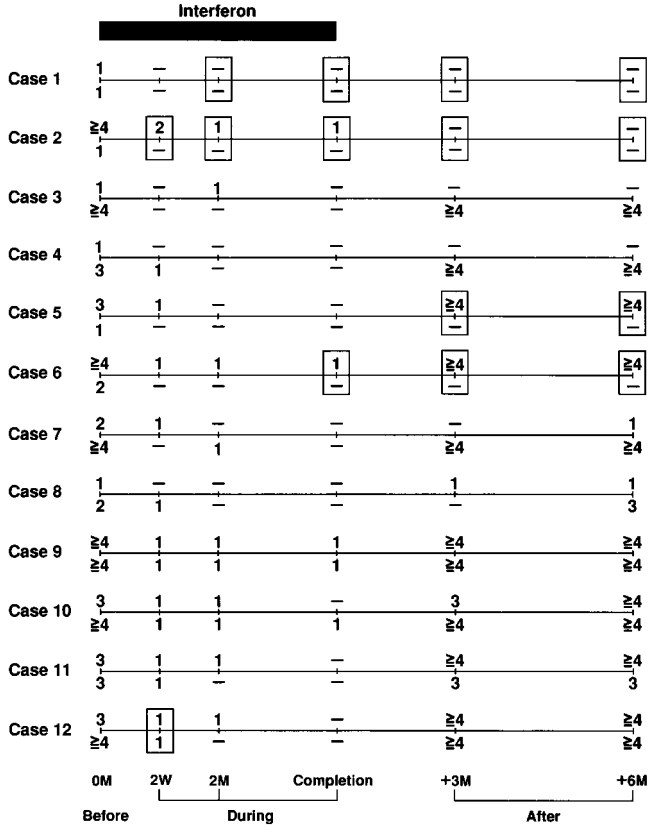

Fig. 1. Titers of GB virus C and hepatitis C virus RNAs, as well as normalization of alanine aminotransferase levels in the 12 patients who received interferon. Results of determinations before, during, and after interferon therapy are shown. Titers of GBV-C RNA (10^N/ml) are indicated above the line and those of HCV RNA below. Open boxes represent normal alanine aminotransferase levels (4–17 IU/L).

Responders to IFN had low pretreatment titers of HCV RNA (10^1–10^2/ml), and 3 were infected with HCV

of genotype III/2a; the remaining patient was infected with II/1b. Overall, the response was achieved by 3 (50%) of the 6 patients infected with genotype III/2a and by 1 (25%) of 4 patients with II/1b.

DISCUSSION

Of the 74 patients with chronic hepatitis C who received IFN, GBV-C RNA was detected by RT-PCR with nested primers derived from the 5′UTR [Shimizu et al., 1996] in 12 (16%) patients at a rate much higher than in 4 (0.9%) of 448 blood donors in Japan [Masuko et al., 1996]. GBV-C tended to co-infect with HCV, in agreement with previous reports [Linnen et al., 1996; Masuko et al., 1996; Tsuda et al., 1996]. After the completion of IFN therapy, 4 lost GBV-C RNA and remained negative for at least 6 months, while 4 remained clear of HCV RNA during this period with the same response rate; 2 were persistently negative both for GBV-C and HCV RNAs. Hence GBV-C would respond to IFN independently of HCV with a comparable efficacy.

Cases of community-acquired and posttransfusion non-A to E hepatitis have mostly a mild subclinical course [Alter and Bradley, 1995]. There are, however, patients with cryptogenic cirrhosis in whom the cause of disease cannot be specified and in whom non-A to E hepatitis virus is implicated. GBV-C/HGV seems to be disseminated worldwide, with prevalence rates among blood donors ranging from 1 to 2% [Linnen et al., 1996; Masuko et al., 1996]. Recently, GBV-C RNA has been reported in 11 (35%) of the 31 subjects with acute hepatitis and 7 (39%) of the 18 patients with chronic hepatitis of unknown etiology in Italy [De Lamballerie et al., 1996]. Moreover, GBV-C RNA has been detected in 3 of 6 patients with fulminant non-A to E hepatitis [Yoshiba et al., 1995].

Since all the patients infected with GBV-C in the present study were infected with HCV as well, the effect of infection with GBV-C alone is not explored. From the comparison between the patients with chronic hepatitis C who were infected with GBV-C and those who were not, GBV-C does not seem to exert any additional hepatitis-inducing activity, supporting previous reports [Masuko et al., 1996; Tsuda et al., 1996]. This view would be supported by the persistence of GBV-C RNA in patients with chronic hepatitis C who cleared serum HCV RNA and whose ALT levels remained normal.

It is not known whether or not HCV possesses any cytotoxicity on hepatocytes. There are accumulating lines of evidence to indicate that like HBV, HCV would also induce hepatitis through cellular immune responses of the host against HLA-restricted epitopes [Koziel et al., 1992; Ferrari et al., 1994; Chisari and Ferrari, 1995]. In treatment of patients with chronic hepatitis B, IFN has been indicated only for those with active disease and elevated transaminase levels [Wong et al., 1993]. Likewise, IFN is not indicated for symptom-free carriers of HCV at present [Serfaty et al., 1996]. Hence IFN might work preferentially on active disease induced by hepatitis viruses. Such a concept would have to be evaluated in patients with chronic hepatitis who are infected with GBV-C/HGV, as reported recently [Fiordalisi et al., 1996].

Until 30 years ago, only 2 hepatitis viruses were known. Now the total of hepatitis viruses is 6. Some of these viruses tend to co-infect, and are typified by HBV and hepatitis delta virus, and perhaps by GBV-C and HCV. Indeed, one has to deal with more than one virus in the care of hepatitis patients. Co-infection may induce severe disease, such as co-infection with hepatitis delta virus and HBV, while others interfere, as in the case of mixed infection with HCV and HBV [Purcell, 1994]. Because of frequent co-infection, the interaction between GBV-C and HCV is a matter of concern. Titers of HCV RNA in the 12 patients who were co-infected were a little lower than those in the 62 patients who were not, suggesting some interference between GBV-C and HCV. This needs to be evaluated in an extended series of co-infected individuals and patients.

The only difference between patients co-infected with GBV-C and HCV and those infected with HCV only was that the age of the former was being significantly higher than the age of the latter. This would indicate that the risk of GBV-C infection increases with age in HCV carriers. Known parenteral risk factors for virus transmission such as transfusions and tattooing were no different between the two groups; they were identified in one-third of each. There would be other routes of transmission for GBV-C than the defined exposure, as is the case for HCV [Alter et al., 1990]. More data are needed to understand the role of GBV-C/HGV in inducing hepatitis.

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